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The changes in the endothelial expression of cell adhesion molecules and iNOS in the vessel wall after the short-term administration of simvastatin in rabbit model of atherosclerosis.

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Abstract

Cell adhesion molecules P-selectin, VCAM-1 and ICAM-1 play an important role in the pathogenesis of atherosclerosis. High levels of nitric oxide (NO) produced by inducible NO synthase (iNOS) have been associated with atherosclerotic processes. Simvastatin is an HMG-CoA reductase inhibitor responsible for many clinical benefits. The aim of this study was to detect and quantify changes in endothelial expression of P-selectin, VCAM-1, ICAM-1 and iNOS in the vessel wall after the short-term administration of simvastatin in a rabbit model of atherosclerosis. Eighteen New Zealand White rabbits were randomly divided into three groups ($n = 6$). In the cholesterol group, rabbits consumed an atherogenic diet (0.4% cholesterol) for eight weeks. In the simvastatin group, rabbits consumed an atherogenic diet for six weeks and then consumed an atherogenic diet supplemented with simvastatin (10 mg kg^{-1}) for two weeks. Biochemical analysis showed that administration of simvastatin led to an almost two-fold lowering of the total serum cholesterol, VLDL, LDL and HDL, but not triglycerides, compared with the cholesterol-fed rabbits only. Stereological analysis of the immunohistochemical staining revealed that administration of simvastatin (10 mg kg^{-1} daily) in an atherogenic diet decreased the endothelial expression of P-selectin, ICAM-1 and iNOS in both aortic arch and carotid artery compared with the cholesterol fed-rabbits only. We conclude that simvastatin has beneficial effects on endothelial function by decreasing expression of P-selectin, ICAM-1 and iNOS in endothelial cells in the very early stages of atherogenesis.

Introduction

The role of cell adhesion molecules (CAMs) has been intensively studied during the past decade. These molecules have received much attention, not only for their participation in normal physiological processes, but also for their potential role as modulators of uncontrolled cell–cell interactions that contribute to vascular dysfunction and tissue damage associated with different vascular diseases (Krieglstein & Granger 2001). The cell-surface expression of several of these molecules in response to pathophysiological stimuli mediates the interaction between the endothelium and blood cells central to the development of atherosclerosis. More recently, these molecules have been shown to participate in cell migration, signalling functions, and other vascular physiological responses (Price & Loscalzo 1999). Platelet selectin (P-selectin), vascular cell adhesion molecule (VCAM-1) and intercellular cell adhesion molecule (ICAM-1) are expressed by activated endothelial cells. Furthermore, P-selectin is expressed by platelets and VCAM-1 and ICAM-1 are expressed by phenotypically modulated smooth muscle cells and by macrophages. These cell adhesion molecules play an important role in the very early stages of atherogenesis (Jang et al 1994; Sakai et al 1997). Additionally, these adhesion molecules participate even in the inflammatory reaction in more advanced atherosclerotic lesions, where they are expressed by intimal cells and endothelial cells of intimal neovessels in atherosclerotic plaques (O'Brien et al 1996). Thus, P-selectin, VCAM-1 and ICAM-1 are crucial for the recruitment of leucocytes and subsequent

formation of foam cells in atherosclerotic lesions at all stages of atherosclerosis. The production of nitric oxide (NO) is necessary for the physiological function of the endothelium. In addition to the regulation of vascular tone, NO regulates leucocyte adhesion to the endothelium, inhibits vascular smooth muscle cell proliferation and also inhibits platelet aggregation (Hotta et al 1999). However, endothelial cell NO may not only preserve normal endothelial integrity but, at elevated levels, can also induce injury to the endothelium. Thus, endothelial NO can play a dual role in atherogenesis (Toborek & Kaiser 1999). High levels of NO sufficient to induce cell injury may be produced by inducible nitric oxide synthase (iNOS) in response to cytokine stimulation.

3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (i.e., simvastatin, lovastatin, pravastatin, etc.), commonly referred to simply as statins, are widely used drugs for the treatment of clinical manifestations and complications of atherosclerosis. The major mechanism of the statins is the inhibition of cholesterol synthesis in the liver by blocking the conversion of HMG-CoA to mevalonate, the rate-limiting step in cholesterol biosynthesis. Simvastatin is a lipophilic widely used statin, which is responsible for unquestionable clinical benefits (Zou et al 2003). Several studies have shown that simvastatin treatment can affect the levels of soluble cell adhesion molecules (Ceriello et al 2004; Koh et al 2004). In addition, it has been shown that statins are able to attenuate the oxidative stress that is associated with NO metabolites by reduction of iNOS expression in the atherosclerotic lesion (Alfon et al 1999; Thakur et al 2001). However, little is known about the effect of simvastatin on endothelial expression of cell adhesion molecules and iNOS in the vessel wall in the very early stages of atherogenesis. Therefore, the aim of this study was to detect and quantify changes in endothelial expression of P-selectin, VCAM-1, ICAM-1 and iNOS in the vessel wall after the short-term administration of simvastatin in a rabbit model of atherosclerosis.

Materials and Methods

Animals and diet

Eighteen adult male New Zealand White rabbits, 2–3 kg (BIOTEST Konarovice, Czech Republic), were housed individually in cages and quarantined for 7 days before the experiment began. They were divided randomly into three groups. In the cholesterol group ($n = 6$), rabbits consumed an atherogenic diet (0.4% cholesterol, 3% fat, 19% proteins) for eight weeks. In the simvastatin group ($n = 6$), rabbits consumed the same atherogenic diet for six weeks and then consumed an atherogenic diet supplemented with simvastatin for two weeks. The simvastatin (kindly provided by Dr Jiri Havranek, Zentiva, Czech Republic) was administered orally at a dose of $10\,mg\,kg^{-1}$ per day. In the control group ($n = 6$), rabbits consumed a standard diet for eight weeks. Rabbits received 100 g of the diet per day, and the food intake was monitored every day. During the course of the experiment, the rabbits were kept under 12–12-h light–dark standard conditions and allowed free access to water. No differences in average weekly food consumption and body weight during the experiment were found among the rabbits. At the end of the experiment, the rabbits were anaesthetized with an intramuscular dose of ketamine ($1\,mL\,kg^{-1}$) and then were euthanized with an overdose of sodium pentobarbital (50 mg/1 mL of distilled water) administered into the auricular vein. Rabbits were bled from the central artery of the ear and blood was collected in tubes containing $1\,mg\,mL^{-1}$ ethylenediaminetetraacetic acid (EDTA). Serum lipoprotein fractions were prepared using NaCl density gradient ultracentrifugation (Beckman TL 100; Palo Alto, CA). The lipoprotein fractions were distinguished in the following density ranges: very-low-density lipoprotein (VLDL) $< 1.006\,g\,mL^{-1}$; low-density lipoprotein (LDL) $< 1.063\,g\,mL^{-1}$; high-density lipoprotein (HDL) $> 1.063\,g\,mL^{-1}$. Total concentration and lipoprotein fraction concentration of cholesterol were assessed enzymatically by conventional diagnostic kits (Lachema, Brno, Czech Republic) and spectrophotometric analysis (cholesterol at 510 nm, triglycerides at 540 nm wavelength) (ULTROSPECT III; Pharmacia LKB Biotechnology, Uppsala, Sweden).

The Ethical Committee of the Faculty of Pharmacy, Charles University approved the protocols of the animal experiments. The protocol of experiments was pursued in accordance with the directive of the Ministry of Education of the Czech Republic (No. 311/1997).

Immunohistochemistry

The immunohistochemical and stereological analysis was performed in 1-cm-long segments of the aortic arch and left carotid artery. Segments of aorta were immersed in OCT compound, snap frozen in liquid nitrogen, and stored at −80°C. Serial aortic cross-sections (7 μm) were cut on a cryostat and placed on gelatin-coated slides. Sections were air-dried and then slides were fixed for 20 min in acetone at −20°C. Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide in phosphate-buffered saline (PBS) (15 min). After blocking of nonspecific binding sites with 10% normal goat serum (Sigma-Aldrich Chemie, Steinheim, Germany) in PBS solution (pH 7.4) for 30 min, slides were incubated with primary antibodies for 1 h at room temperature. After a PBS rinse, the slides were developed with a secondary antibody – goat anti-mouse IgG conjugated to peroxidase-labelled polymer (DAKO EnVision$^+$, Carpinteria, USA). Bound antibodies were visualized with diaminobenzidine (DAB substrate-chromogen solution; DAKO, Carpinteria, USA). As a control for background staining, control slides were treated in the same manner, except PBS solution was substituted for the primary antibodies.

Primary antibodies included the following: monoclonal mouse anti-rabbit VCAM-1 (Rb1/9, IgG1) and monoclonal mouse anti-rabbit ICAM-1 (Rb2/3, IgG1) diluted 1:10 and 1:20, respectively, that were a generous gift from Dr M. I. Cybulsky (University of Toronto, Toronto, Canada); monoclonal mouse anti-human CD31 (PECAM-1, JC/70A, IgG_1; DAKO, Carpinteria, USA);

ready-to-use monoclonal mouse anti-human P-selectin (GMP-140, clone NPL44-10, IgG1; Takara Bio Inc., Shiga, Japan) diluted 1:400; and monoclonal mouse anti-human iNOS diluted 1:200 (clone 54, IgG_1; BD Transduction Laboratories, San Jose, USA). Mouse antibody cross-reacts with rabbit activated endothelial cells (Hoshida et al 1999).

Quantitative analysis of immunohistochemistry

Stereological methods for the estimation of immunohistochemical staining of P-selectin, VCAM-1, ICAM-1, PECAM-1 and iNOS were used as previously described (Nachtigal et al 2002, 2004). In brief, the systematic uniform random sampling and the principle of the point-counting method were used for the estimation (Weibel 1979). A total number of 55 consecutive serial cross-sections were cut into 7-μm-thick slices, which gave us 0.385-mm-long pieces of the vessel, called the reference volume. A systematic uniform random sampling in the reference volume was used. The first section for each immunohistochemical staining was randomly positioned in the reference volume and then each 11th section was used; thus five sections for each staining were used for the stereological estimation. The point-counting method was used and more than 200 test points hitting either immunostaining or the atherosclerotic lesion per vessel were counted for an appropriate estimation (Gundersen et al 1988). The estimated area is then:

$$\mathrm{EstA} = \mathrm{a} \times \mathrm{P} \quad (1)$$

where the parameter a characterizes the test grid and P is the number of test points hitting either atherosclerotic lesion or positive immunostaining.

The area of PECAM-1 expression was considered as a total area of intact endothelium. Thus, the area of P-selectin, VCAM-1, ICAM-1 and iNOS expression indicates the percentage of activated endothelial cells calculated as:

$$\mathrm{EstP} = \mathrm{area(x)/area(PECAM)} \times 100\% \quad (2)$$

where area (x) is the area of P-selectin, VCAM-1, ICAM-1 or iNOS, respectively, in the endothelium and area (PECAM) is the area of PECAM-1 in the endothelium.

Photodocumentation and image digitizing from the microscope were performed with the Nikon Eclipse E2000 microscope, with a digital firewire camera Pixelink PL-A642 (Vitana Corp., Ottawa, Canada) and with image analysis software LUCIA version 4.82 (Laboratory Imaging Prague, Prague, Czech Republic). Stereological analysis was performed with a PointGrid module of the ELLIPSE software (ViDiTo, Kosice, Slovakia).

Statistical analysis

All values in the graphs are presented as the mean $\pm$ s.e.m. of n = 6 rabbits. Statistical significance in the differences between groups was assessed by one-way analysis of variance followed by the Tukey test for multiple comparisons with the use of the SigmaStat software (version 3.0). $P \leq 0.05$ was considered statistically significant.

Results

Biochemical analysis

Biochemical analysis showed a significant increase in the total serum cholesterol, VLDL, LDL and HDL in the cholesterol and simvastatin groups compared with the control group ($P < 0.05$). Administration of simvastatin led to an almost two-fold lowering of the total serum cholesterol, VLDL, LDL and HDL. Level of triglycerides (TAG) slightly increased in the cholesterol and simvastatin group compared with the control group. However, no difference in TAG level was observed between the cholesterol and simvastatin group (Figure 1).

Immunohistochemical staining in the aortic arch

PECAM-1 expression was observed only in endothelial cells in all groups of rabbits and this antibody was used as standard for the detection of intact endothelium (data not shown). There were no atherosclerotic lesions in the control group of rabbits. Furthermore, the expression of VCAM-1, P-selectin and iNOS was very low in comparison with the cholesterol group. On the contrary, there was strong endothelial expression of ICAM-1 in aortic arch in control rabbits (data not shown).

The fibromuscular type of lesion was observed only in the simvastatin and cholesterol group. In the cholesterol group, the expression of P-selectin, VCAM-1, ICAM-1 and iNOS was detected in endothelial cells of early lesions and even in endothelium where no lesions were found (Figure 2). In addition, strong ICAM-1 and VCAM-1 expression was observed in the intimal cells of the lesions (Figure 2A, B, C, D). ICAM-1 expression was spread over the whole lesion (Figure 2A, B). VCAM-1 was abundant at the base of the lesion (near the lamina elastica interna) and in the medial smooth muscle cells adjacent to the lesions (Figure 2C, D). Two-week administration of

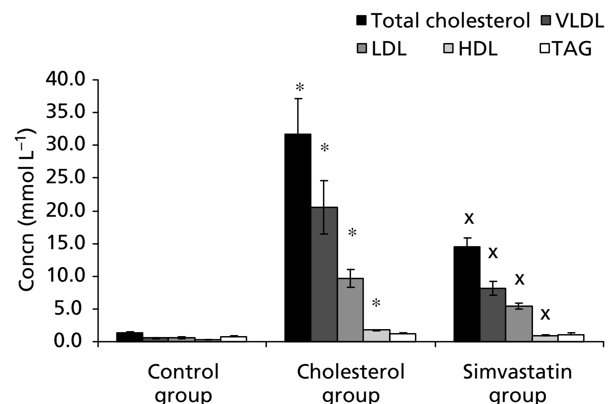

Figure 1 Total serum cholesterol levels, VLDL, LDL, HDL and TAG. *$P < 0.05$ compared with control rabbits; $^{x}P < 0.05$ compared with the control rabbits. No statistically significant difference was observed between the cholesterol and simvastatin group. Note that cholesterol is located predominantly in VLDL in all groups of rabbits.

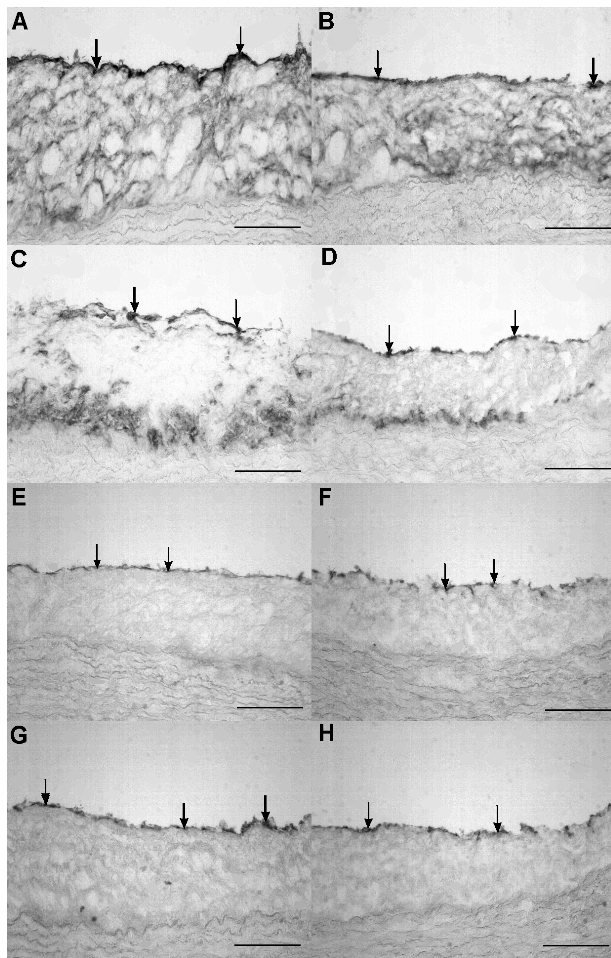

Figure 2 The expression of ICAM-1 (A, B) and VCAM-1 (C, D) in the aortic arch in the cholesterol (A, C) and simvastatin group (B, D) of rabbits. Both ICAM-1 and VCAM-1 are expressed in endothelium of the lesion (arrows). Note the strong expression of ICAM-1 in intimal cells of the lesion and the strong expression of VCAM-1 at the base of the lesion in both the cholesterol and the simvastatin group. The expression of P-selectin (E, F) and iNOS (G, H) in the aortic arch in the cholesterol (E, G) and simvastatin group (F, H). Both P-selectin and iNOS are expressed only in endothelium of the lesion (arrows). Note the stronger endothelial staining of iNOS (G, H) compared with the P-selectin staining (E, F). Bar = 50 μm.

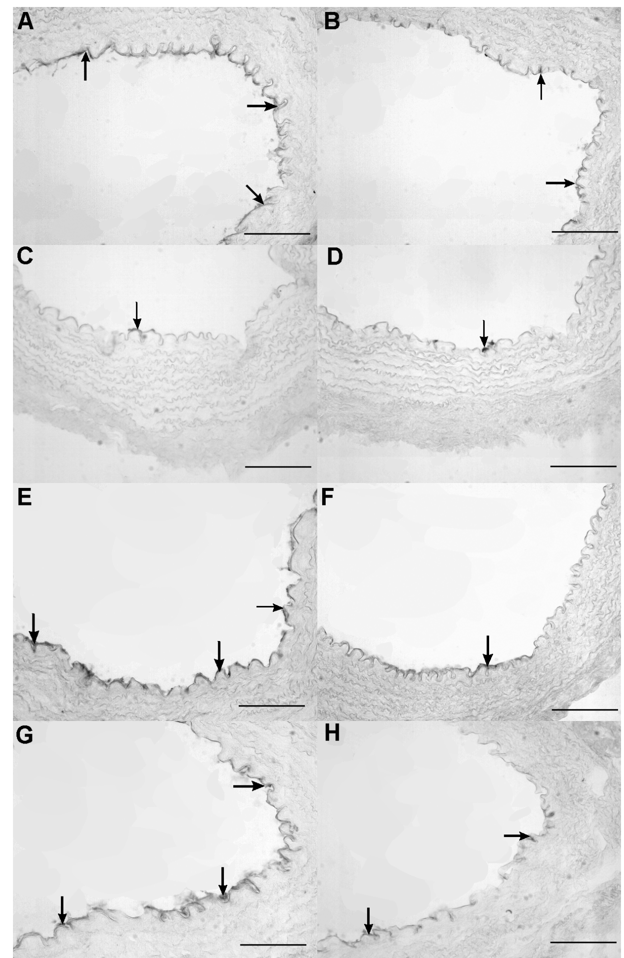

Figure 3 The expression of ICAM-1 (A, B) and VCAM-1 (C, D) in the carotid artery in the cholesterol (A, C) and simvastatin group (B, D) of rabbits. Note the weak VCAM-1 staining located only in several endothelial cells in both experimental groups. The expression of P-selectin (E, F) and iNOS (G, H) in the carotid artery in the cholesterol (E, G) and simvastatin group (F, H). Note the strong diminution of ICAM-1, P-selectin and iNOS expression in the simvastatin group (B, F, H) compared with the cholesterol group. Bar = 50 μm.

simvastatin together with the atherogenic diet led to changes in the endothelial expression of P-selectin, ICAM-1 and iNOS in the simvastatin group. The endothelial expression of P-selectin, ICAM-1 and iNOS was slightly weaker in the simvastatin group (Figure 2). On the contrary, the endothelial expression of VCAM-1 remained unaffected by the simvastatin treatment (Figure 2D).

Immunohistochemical staining in the carotid artery

No atherosclerotic lesions were found in the left carotid artery in both experimental and control groups of rabbits. Weak endothelial expression of ICAM-1, P-selectin and iNOS was observed in the control group (data not shown). On the contrary, strong endothelial expression of all studied adhesion molecules, except for VCAM-1, was observed in the cholesterol group (Figure 3). VCAM-1 expression was detected only in several endothelial cells (Figure 3C). Simvastatin treatment markedly decreased endothelial expression of ICAM-1 and P-selectin, as well as iNOS, in comparison with the cholesterol group (Figure 3B, F, H). Furthermore, endothelial expression of P-selectin was weaker in the cholesterol group in comparison with the control group (data not shown). VCAM-1 expression was a little bit stronger in the simvastatin group compared with the cholesterol group, but it was still visible only in a few endothelial cells (Figure 3D).

Stereological analysis of cell adhesion molecule expression in the vessel wall

The expression of ICAM-1, P-selectin, VCAM-1 and iNOS in endothelium was related to the PECAM-1 staining of the endothelium and thus the results indicate the percentage of activated endothelial cells. Stereological analysis confirmed that the cholesterol diet increased endothelial expression of all studied adhesion molecules in both aortic arch and carotid arteries, except for ICAM-1 in the aortic arch, compared with the control group. Endothelial expression of ICAM-1 in the aortic arch was even a little stronger in the control group than in the cholesterol group (Figure 4). Moreover, the stereological analysis revealed that simvastatin treatment decreased the endothelial expression of ICAM-1, P-selectin and iNOS in both aortic arch and left carotid artery compared with the cholesterol-fed rabbits only (Figures 4 and 5). The strongest diminution of endothelial expression was observed for iNOS in the aortic arch. On the contrary, VCAM-1 staining was almost the same in the aortic arch in the cholesterol and simvastatin groups. The diminution of endothelial expression of ICAM-1, P-selectin and iNOS was more marked after the simvastatin treatment in the carotid artery compared with the aortic arch (Figure 5). However, statistically significant diminution was observed only in P-selectin expression in the simvastatin group compared with the cholesterol group (Figure 5). The VCAM-1 endothelial expression was very weak in both the cholesterol and the simvastatin group and, furthermore, no expression was detected in the control rabbits (Figure 5).

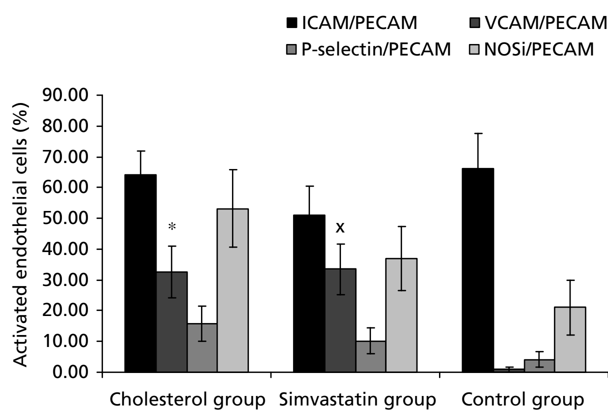

Figure 4 The percentage of activated endothelial cells in the aortic arch of rabbits. $*P < 0.05$ compared with the control group; $^{x}P < 0.05$ compared with the control group.

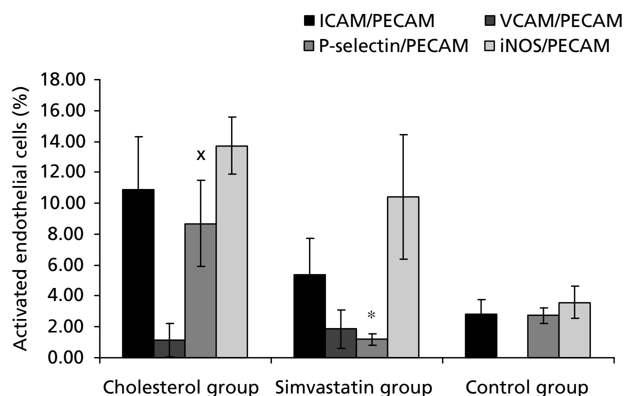

Figure 5 The percentage of activated endothelial cells in the left carotid artery of rabbits. $*P < 0.05$ compared with the cholesterol group; $^{x}P < 0.05$ compared to the control group.

Discussion

Clinical trials of HMG-CoA reductase inhibitor therapy demonstrate an improvement in cardiovascular end points and coronary stenosis (Mark & Katona 2000; Martin Lujan et al 2004). Statins competitively inhibit HMG-CoA reductase, the enzyme that catalyses the rate-limiting step in cholesterol biosynthesis. Simvastatin is a lipophilic statin with many clinical benefits. Besides the reduction of LDL in the circulation, simvastatin treatment results in a significant improvement in endothelial function via increased production of endothelial NO synthase (Laufs et al 1998). Additionally, the anti-inflammatory effects of statins were studied. Several authors have shown that simvastatin treatment affects the levels of soluble adhesion molecules (Rezaie-Majd et al 2003; Ceriello et al 2004). However, very little is known about the effect of simvastatin on endothelial expression of cell adhesion molecules in the vessel wall in the very early stages of atherogenesis.

In this study, we focused on the expression of P-selectin, VCAM-1, ICAM-1 and iNOS in the vessel wall after short-term administration of simvastatin in a high-cholesterol diet. It is well established that interactions of endothelial cells and leucocytes via cell adhesion molecules play an important role in leucocyte recruitment in atherogenesis (Jang et al 1994). The role of P-selectin, VCAM-1 and ICAM-1 has largely been studied in rabbit and mouse models of atherosclerosis (Sakai et al 1997). It has been shown that the expression of these adhesion molecules is up-regulated by a high-cholesterol diet (Cybulsky et al 1999). Furthermore, increased levels of soluble forms of cell adhesion molecules in blood from patients with atherosclerosis and dyslipidaemia have been observed (Blann & McCollum 1994). Thus, it is obvious that hypercholesterolaemia increases the expression of P-selectin, VCAM-1 and ICAM-1 in both man and experimental animals.

In this study, the endothelial expression of P-selectin and VCAM-1 was strongly up-regulated in cholesterol-fed rabbits in the aortic arch in comparison with control rabbits. On the contrary, ICAM-1 endothelial expression was almost the same in the aortic arch of control and cholesterol-fed rabbits. These results are consistent with the previous reported data by Sakai et al (Sakai et al 1997). Moreover, endothelial expression of P-selectin and ICAM-1 was up-regulated in the carotid arteries of cholesterol-fed rabbits.

Stereological analysis of the immunohistochemistry revealed that administration of simvastatin with an atherogenic diet decreased the endothelial expression of ICAM-1 and P-selectin. It was found that diminution of endothelial expression of these markers was stronger in the carotid arteries where no atherosclerotic lesion was found. Thus, it seems that the influence of simvastatin on the endothelium might be more effective before the formation of the atheroclerotic lesion. Pruefer et al (1999, 2002) showed that simvastatin is able to attenuate both L-NAME and thrombin-induced leucocyte–endothelial cell interactions or acute inflammatory states induced by the exotoxin *Staphylococcus aureus* a-toxin via a P-selectin-dependent mechanism. However, our results are the first to demonstrate that simvastatin is able to reduce the endothelial expression of P-selectin and ICAM-1 in atherosclerosis-prone regions in the vascular tree and in the very early stages of atherogenesis. On the contrary, endothelial expression of VCAM-1 was unaffected by the simvastatin treatment both in the aortic arch and carotid artery. However, Sukhova et al (2002) showed that VCAM-1 expression was lower after statin treatment in non-human primates. Furthermore, Aikawa et al (2002) demonstrated that VCAM-1 expression was decreased by dietary lipid lowering in rabbits. As mentioned above simvastatin treatment had less effect in the aortic arch. Furthermore, VCAM-1 expression was very weak in the carotid artery, both in the cholesterol and simvastatin groups, in comparison with the expression of the other markers studied. Thus, we suggest that simvastatin is likely to be able to decrease the expression of VCAM-1, although we were not able to confirm that in our study.

It should be stated that not only hypercholesterolaemia is responsible for an up-regulation of these cell adhesion molecules. Other inflammatory markers, such as IL-1, TNF-alpha or high oxidative stress, may participate in their expression (Aikawa et al 2002; Zapolska-Downar et al 2004). Increased oxidative stress in endothelium can be induced by the overproduction of NO, which is produced by iNOS (White et al 1994). NOS II is the inducible isoform of NOS in macrophages, vascular smooth muscle cells and other cells and tissues, which produce NO. Esaki et al (1997) and Behr et al (1999; Behr-Roussel et al 2000) reported the presence of iNOS in advanced atherosclerotic plaques, but not in normal vessels or the early stage of atherosclerosis. Moreover, they showed that iNOS is produced especially in the atherosclerotic intima by macrophages and T lymphocytes. Surprisingly, we found strong iNOS expression only in endothelium covering fatty streaks, in fibromuscular lesions in the aortic arch, and even strong expression only by endothelium in carotid arteries where no atherosclerotic lesions were found. In addition, no iNOS staining was visible in other areas of the atherosclerotic intima. Thus, our results are consistent with those of Yang & Chen (2003), who described predominant iNOS staining in vascular endothelium. It has been established that HMG-CoA reductase inhibitors up-regulate the expression and activity of endothelial NOS (eNOS) in rabbit atherosclerotic lesions, which leads to the restoration of NO production by endothelium and thus restoration of endothelial dysfunction (Kolyada et al 2001; Thakur et al 2001). On the contrary, it has been shown that statins are able to attenuate the oxidative stress that is associated with NO metabolites by reduction of iNOS expression in the atherosclerotic lesion (Alfon et al 1999; Thakur et al 2001). In our study, we demonstrated that simvastatin decreases endothelial expression of iNOS in fibromuscular lesions in the aortic arch as well as in carotid arteries were no lesions were found. Thus, we suggest that statins attenuate the oxidative stress in the vessel wall by reducing iNOS expression not only in macrophages or T lymphocytes in advanced atherosclerotic lesions but also even in endothelial cells in very early stages of atherogenesis.

It must be stated that there are possible limitations of this study. Firstly, the results of the biochemical and stereological analysis clearly show that the short-term administration of simvastatin has a beneficial effect. However, the small number of rabbits and strong variance of the values result in many statistically insignificant outcomes. Moreover, we were not able to determine whether decreased endothelial expression of ICAM-1, P-selectin and iNOS is dependent or independent of the lipid-lowering effect of simvastatin. Thus, further study using an apo-E knockout mouse model of atherosclerosis might be used because the critical feature of these models is that simvastatin does not affect plasma lipid levels, and therefore the results may be interpreted without this confounding variable (Sparrow et al 2001).

Conclusion

In conclusion, we demostrated in rabbits that a two-week administration of simvastatin ($10\,mg\,kg^{-1}$ per day) during an atherogenic diet had a strong lipid-lowering effect and, moreover, it resulted in diminution of endothelial expression of P-selectin, ICAM-1 and iNOS in both the aortic arch and carotid artery compared with cholesterol-fed rabbits only. It is well known that up-regulation of cell adhesion molecule expression, together with increased oxidative stress, is one of the markers of endothelial dysfunction (Toborek & Kaiser 1999). Thus, we suggest that simvastatin has beneficial effects on endothelial function not only by reducing serum cholesterol levels, or via increased production of endothelial NO (Laufs et al 1998), but also via decreased expression of P-selectin, ICAM-1 and iNOS in endothelial cells in the very early stages of atherogenesis.

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